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SPROUTING AND PLASTICITY
OF CENTRAL MONOAMINE NEURONS
WITH MICROSPECTROFLUOROMETRIC
CHARACTERIZATION OF THEIR
INTRANEURONAL FLUOROPHORES

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From the Institute of Anatomy and Histology

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To C. Brown

June 1971

The present summary is based on the following papers:

- I On the possible existence of a new intraneuronal monoamine in the spinal cord of the rat. *J. Pharmacol. Exp. Ther.* 1970. 175. 525-532. (Together with A. Björklund and B. Falck).
- II Classification of monoamine neurones in the rat mesencephalon: Distribution of a new monoamine neurone system. *Brain Research*. In press. (Together with A. Björklund and B. Falck).
- III Microspectrofluorimetric characterization of monoamines in the central nervous system: Evidence for a new neuronal monoamine-like compound. *Progress in Brain Research*. In press. (Together with A. Björklund and B. Falck).
- IV Evidence for regenerative axon sprouting of central catecholamine neurons in the rat mesencephalon following electrolytic lesions. *Brain Research*. 1971. 25. 579-596. (Together with R. Katzman, A. Björklund, Ch. Owman and K. West).
- V Growth of central catecholamine neurons into smooth muscle grafts in the rat mesencephalon. *Brain Research*. In press. (Together with A. Björklund).
- VI Development and growth of axonal sprouts from noradrenaline and 5-hydroxytryptamine neurons in the rat spinal cord. *Brain Research*. In press. (Together with A. Björklund, R. Katzman and K. West).
- VII Plastic changes in the adrenergic innervation of the rat septal area in response to denervation. *Brain Research*. In press. (Together with R.Y. Moore and A. Björklund).

These papers will be referred to by their Roman numerals.

INTRODUCTION

The histofluorescence method for the visualization of certain biogenic monoamines was devised about ten years ago by Falck, Hillarp and co-workers (Falck and Torp 1961, Falck 1962, Falck et al. 1962, Corrodi and Hillarp 1963, 1964) and has been used extensively in the study of central and peripheral monoamine-containing cell systems. The chemical background of the fluorophore formation in this method, involving a condensation reaction between the monoamine (or amino acid) and formaldehyde with a subsequent dehydrogenation, is now well known (Corrodi and Hillarp 1963, 1964, Corrodi and Jonsson 1965, Jonsson 1966, for review see Corrodi and Jonsson 1967) and the different reaction steps can to a certain extent be manipulated (Björklund et al. 1968b and 1971b, Björklund and Stenevi 1970).

The neuronal localization of catecholamines in the central nervous system was first demonstrated with the histochemical fluorescence method by Carlsson et al. (1962). Later, fundamental studies were presented by Dahlström and Fuxe who described the distribution of monoamine-containing cell bodies and terminals within the CNS of the rat (Dahlström and Fuxe 1964, 1965, Fuxe 1965, Fuxe et al. 1968, 1969). The monoamine pathways in the rat brain have also been investigated (Andén et al. 1966) and recently, a detailed mapping was presented by Ungerstedt (1971). It should be noted that the existence of central monoamine neuron systems was predicted - before a direct visualization of the neuronal monoamines was possible - on the basis of stereotaxic denervation experiments (Heller et al. 1962, cf. Moore 1970).

A critical point in all studies with the Falck-Hillarp technique is the identification of the studied fluorogenic compound, i.e. the compound

that yields fluorescence after the formaldehyde gas treatment. Certain criteria have been developed for this identification, such as the colour of the fluorophore in the fluorescence microscope, the sensitivity of the fluorophore to exposure to ultraviolet light, the reaction conditions necessary to obtain maximum fluorescence, and the effect of certain drugs on the tissue concentration of certain fluorogenic monoamines (cf. Dahlström and Fuxe 1965, Falck and Owman 1965). The development of microspectrofluorometry of monoamine fluorophores in tissue sections (van Orden et al. 1965, Caspersen et al. 1966, Jonsson 1967, Björklund et al. 1968a) has made possible a precise and reproducible characterization and in many cases also identification of the studied fluorogenic substance at its cellular storage site. This new technique thus offered the possibility for a basic characterization of intraneuronal monoamine fluorophores in the central nervous system.

The intra-axonal accumulations of amines in transected axons allow the histochemical demonstration also of the preterminal part of the monoamine neurons (Dahlström and Fuxe 1965, Andén et al. 1966). Such accumulations produced by stereotaxic lesions makes it possible to trace and to map central monoamine neuron systems (Dahlström and Fuxe 1965, Andén et al. 1966, Ungerstedt 1971) and should also permit reliable microspectrofluorometric characterization of the intraneuronal fluorophores. Administration of a monoamine oxidase inhibitor will also increase the intraneuronal 5-hydroxytryptamine fluorescence (Dahlström and Fuxe 1964); these two methods were used alone or in combination in the present series of investigations.

Drugs that interfere with the synthesis, uptake, storage, release, or breakdown of central monoamines can be used as valuable tools in the study of these intraneuronal substances in the brain. Such drugs with specific effects can be used for selective manipulations of the monoamine neuron systems. The effect of certain drugs will also to some extent characterize the intraneuronal fluorogenic compound in any given cell. This particular

approach was also chosen in the effort to characterize central monoamine neurons.

Neuronal regeneration in the central nervous system of mammals has been a controversial subject for many years and has been the object of numerous investigations in which a variety of different techniques have been used (for review, see Windle 1955 and Clemente 1964). Although many authors (Davidoff and Ransohoff 1948, Bernard and Carpenter 1950, Feigen et al. 1951, Lance 1954) deny that regeneration takes place in the central nervous system, others take the view, supported by Cajal (1928), that a regenerative process starts from the severed axons, but is followed by atrophy and resorption after four to five weeks. Many attempts have been made to influence this initial sprouting process (Gokay and Freeman 1952, Windle et al. 1952, Scott and Clemente 1955, Arteta 1956, Clemente 1958, Harvey and Srebnik 1967), but these treatments seem to effect mainly the formation of scar tissue rather than the actual sprouting itself. These and similar experiments supported the idea that the regenerative capacity of the central nervous system is small compared with that of the peripheral nervous system and several possible explanations for this difference have been proposed (cf. Clemente 1964).

In more recent studies, new techniques have provided good evidence that significant sprouting can take place in at least certain neuron systems within the central nervous system (Rose et al. 1960, Goodman and Horel 1967, Raisman 1969). This could be taken to indicate that failure to detect peristant sprouting in the central nervous system of mammals could be due to insensitive and inadequate methods rather than lack of actual sprouting. The very sensitive histofluorescence method of Falck and Hillarp, in combination with microspectrofluorometric analysis, makes it possible to selectively study and compare the regenerative capacity of identified neuron systems. With these methods, regeneration and the response to denervation of certain monoamine systems were studied in rat brain.

MATERIAL AND METHODS

Adult albino rats of the Sprague-Dawley strain were used throughout. In case of transplantation experiments, when the donor and the host were not the same animal, rats were obtained from a strain inbred for more than fifty generations.

Operative procedures

The rats were anaesthetized with ether, sodium pentobarbital, or a mixture of halothane, N_2O , and O_2 . Surgery was performed under clean but not aseptic conditions. Stereotaxic lesions. The head of the animal was fixed in a stereotaxic apparatus and a suitable hole was drilled in the skull. Electrolytic lesions were performed with insulated electrodes that had a small exposed tip (Paper IV). Frontal cuts were produced unilaterally with a micro knife (Papers II, V, and VII). The knife had a horizontal edge and was lowered vertically in the frontal plane through the brain substance to the base of the brain, thus making a frontal lesion, whose width depended on the number of cuts made laterally to one another.

Transplantations. (Papers V and VI). When iris or striated muscles were used as transplants, the host and the donor were the same animal. To obtain the iris, one eye was removed. The iris was carefully dissected out and washed in saline. The striated muscle taken from the neck of the animal was prepared in thin strands approximately 5 mm long and approximately 0.5 mm thick. Uterus, diaphragm, and mitral valve were transplanted from one inbred animal to another. One whole mitral valve was used, and uterus and diaphragm were prepared in thin strands, approximately 5 mm long and approximately 0.5 mm thick. Transplants to the brain. The head of the animal was secured in the head holder of a stereotaxic instrument. A hole was

drilled in the parietal bone and the dura carefully opened. The transplants, washed in saline, were then lowered by free hand through the hole in the dura and into the brain with a small glass tube. The glass tube was slowly removed leaving the transplant in the desired position within the brain. The positions of lesions and transplants were calculated according to the atlas of König and Klippel (1963). Transplants to the spinal cord. The transplants were prepared as described above. A midline incision was made in the skin on the back, and the vertebrae were dissected free. The cord was exposed and a small longitudinal hole was made in the dura on one side of the midline. The transplants, washed in saline, were then transferred through the hole in the dura into the spinal cord with a small glass tube. Spinal cord lesions. The spinal cord was exposed at different levels, as described above. Total transection was performed with a pair of small scissors, and compression of the spinal cord was performed with a pair of small tweezers for 20-30 sec. Only females were used for the compression experiments (cf. Sugar and Gerard 1940) and their urinary bladder was emptied four times daily until bladder function was regained. Hippocampal ablations. (Paper VII). Also these animals had their heads secured in a stereotaxic instrument. A large hole was drilled in the parietal bone on one or both sides, and the medial portion of the hippocampal formation was exposed by aspiration of the overlying cortex and white matter. This part of the hippocampus was then carefully aspirated, thus removing all hippocampal input to the fornix unilaterally or bilaterally. These animals received 25,000 units of penicillin after completed surgery. Cervical sympathectomy. (Papers IV and V) was performed by bilateral removal of the superior cervical ganglia and the effect of the sympathectomy was checked on iris whole mounts.

Drugs.

Atropine suphate (ACO), α -methyl-m-tyrosine (Regis), nialamide (Niamid [®], Pfizer), p-chlorophenylalanine (British Drug Houses), p-chloro-

phenylalanine-metylester hydrochloride (Hässel AB, through Dr. H. Corrodi), penicillin (Benzylpenicillin, Glaxo) and reserpine (Serpasil[®], Ciba).

Histochemical procedures

The animals were killed at times varying between one day and six months after operation. The tissue pieces were rapidly dissected out, frozen in a propene-propylene mixture cooled by liquid nitrogen, freeze-dried, and processed according to the histofluorescence method of Falck and Hillarp (for technical details, see Falck and Owman 1965, Björklund et al. 1971a). The formaldehyde was generated from paraformaldehyde equilibrated in air with approximately 30%, 50%, 70%, or 90% relative humidity (cf. Hamberger 1967). Sections were cut at 5-10 μ and mounted in Entellan (Merck, Darmstadt) or liquid paraffin on glass microscope slides for fluorescence microscopy.

Histochemical models. The substance to be investigated was dissolved in a 2-5% solution of human serum albumin (AB Kabi) or in a 5% solution of sucrose containing 0.1% glycine. The solutions were sprayed on to glass microscope slides or cover slips and were allowed to dry in room temperature. The formaldehyde treatment of the histochemical models was the same as for freeze-dried tissue pieces (see above).

Microspectrofluorometry. Spectral analysis of amine fluorophores in models or in tissue sections was carried out in a modified Leitz[®] microspectrograph as described by Björklund et al. (1968a, 1971a). All spectra are given as corrected instrument values and are expressed as relative quanta vs. wavelength. Photodecomposition rate was recorded at maximum excitation wavelength in the microspectrograph; in all tissue measurement, areas without specific, i.e. formaldehyde induced, fluorescence were used to obtain blank values. In model experiments, identically treated droplets lacking the substance to be investigated were used to obtain blank values.

For further characterization of amine fluorophores, deparaffinized sections were acidified with HCl or alkalinized with NH_3 vapour, or were subjected to a combined formaldehyde and ozone treatment (Björklund et al.

1968a,b, 1971a).

Chemical analysis (Papers IV and VII)

Dopamine in the caudate nucleus was measured according to the method of Bertler et al. (1958). Dopamine and noradrenaline in the septum was analysed by modification of the method of Anton and Sayre (1962, 1964) (Neff and Costa 1966).

The line drawings that formed the basis of the septum diagram (Paper VII) were taken from cresylviolet stained frontal sections from a normal rat brain.

RESULTS AND COMMENTS

1. Classification of central monoamine neurons

Papers I, II and III

Monoamine cell bodies, terminals, and axonal accumulations were observed in the fluorescence microscope and analysed in the fluorescence microspectrograph. In the fluorescence microscope with the commonly used filter setting (secondary filter with high absorption below 490m μ), two types of formaldehyde induced fluorescence could be seen, one green and one yellow. In the microspectrograph, however, three types of intraneuronal fluorophores could be distinguished. The green-fluorescent type showed excitation maxima at about 320 and 410 m μ , and emission maximum at about 475 m μ with a slow photodecomposition rate, characteristic for the fluorophores of the biogenic catecholamines (Paper III; Caspersson et al. 1966, Ritzén 1966a, Corrodi and Jonsson 1967, Jonsson 1967, Björklund et al. 1968a and 1970). The yellow fluorescence could be separated into two distinctly different types. One had the excitation/emission maxima at about 315 and 380-390/520-535 m μ with a rapid photodecomposition rate, characteristic for 5-hydroxytryptamine (Paper III; Caspersson et al. 1966, Ritzén 1966b, Jonsson 1967, Björklund et al. 1968b, Jonsson and Sandler 1969). The other yellow fluorophore showed excitation/emission maxima at about 315 and 370-380 m μ with a shoulder at 420/495-510 m μ , and had a moderate photodecomposition rate. These fluorescence characteristics were not notably affected by drug treatments, operative procedures, or varying humidity of the formaldehyde treatment (Paper I). On the basis of the microspectrofluorometric findings described above, three types of neurons with different intraneuronal fluorophores could

be distinguished. They are characterized by the following criteria:

A-type neurons (catecholamine-containing): excitation/emission maxima at 320 and 410/475 m μ ; slow photodecomposition.

B-type neurons: maxima at 315, 370-380 and 420/495-510; rather slow photodecomposition.

C-type neurons (5-hydroxytryptamine containing): maxima at 315, 380-390/520-535 m μ ; rapid photodecomposition.

Differentiation between the catecholamine fluorophores, the noradrenaline (A-type 1 in Paper III) and the dopamine (A-type 2) fluorophore, can be carried out according to the method of Björklund, Falck and Ehinger (1968), which utilizes the aromatization of the noradrenaline fluorophore at acid pH (Corrodi and Jonsson 1965).

Reaction to drugs. α -methyl-m-tyrosine is known to deplete catecholamines from the central nervous system (Costa et al. 1962 and Carlsson et al. 1964). Nialamide, a monoamine oxidase inhibitor, increases central monoamine levels (Carlsson et al. 1959, Dahlström and Fuxe 1964). p-Chlorophenylalanine, a tryptophane-5-hydroxylase inhibitor, strongly decreases the 5-hydroxytryptamine concentration in the brain and spinal cord, while only slightly reducing the catecholamine content (Koe and Weissman 1966). Reserpine is a well known and often used neuropharmacological agent that depletes endogenous monoamine stores by blocking their intraneuronal storage sites (see Carlsson et al. 1963, Dahlström et al. 1965). The fluorescence intensity of the different fluorescent neuron types was found to vary in response to treatments with the above mentioned drugs in the following way (Papers I and II):

A-type neurons (catecholamine-containing) were depleted by α -methyl-m-tyrosine (only studied on spinal cord specimens, see Paper I) or reserpine, but not notably affected by nialamide or p-chlorophenylalanine.

B-type neurons were depleted by reserpine but were not notably affected by α -methyl-m-tyrosine (only spinal cord, see Paper I) nialamide or p-

chlorophenylalanine.

C-type neurons (5-hydroxytryptamine-containing) were depleted by reserpine or p-chlorophenylalanine, but were not notably affected by α -methyl-m-tyrosine (only spinal cord, see Paper I). Nialamide treatment gave a marked increase in the fluorescence intensity of the C-type neurons.

2. Topography

Papers I and II

A-type fibres (catecholamine-containing) were found in a descending system in the spinal cord, the axons being confined to the white matter, and the terminals distributed mainly within the gray matter, as has been described earlier (Carlsson et al. 1964, Dahlström and Fuxe 1965). In the mesencephalon and caudal hypothalamus, the A-type fibres formed two ascending tracts, one of which was mainly confined to the medial forebrain bundle. This tract could, at least partly, be traced back to cell bodies of the substantia nigra and cells surrounding the anterior part of the interpeduncular nucleus. The other ascending tract of A-type fibres was situated more dorsally (see Paper II) and in the posterior hypothalamus it turned ventrally to join the A-type tract in the medial forebrain bundle. The origin of the dorsal A-type tract was not identified. These findings agree with those reported by Dahlström and Fuxe (1965), Andén et al. (1966) and Ungerstedt (1971).

C-type (5-hydroxytryptamine containing) and B-type fibres were found intermingled in a descending system in the spinal cord. Here the B-type fibres constituted a minor portion of the yellow fluorescent terminals and axonal accumulations. In earlier fluorescence microscopical studies both the B-type and C-type fluorophores have been ascribed to 5-hydroxytryptamine, since the two cannot be safely distinguished in the fluorescence microscope. The distribution of the descending fibres was in good agreement with that reported in previous studies (Carlsson et al. 1964, Dahlström and Fuxe 1965). In the

mesencephalon and caudal hypothalamus the B- and C-type fibres were to some extent intermingled with each other and also with the A-type fibres (Paper II). From a position in the medial forebrain bundle, at the level of the caudal hypothalamus, the B- and C-type fibres could be traced caudally, the B-type having a more medial and the C-type a more lateral position. At least some of the fibres could be followed back to cell bodies in the raphe nuclei, where B-type cell bodies were found predominantly in the caudal lineary nucleus, the median raphe nucleus, and in cells lateral to this nucleus. A minor portion of C-type cell bodies occurred also in these nuclei. The reversed situation, more C- than B-type cell bodies, was found in the dorsal raphe nucleus, and ventrally in the reticular formation (the B9 group of Dahlström and Fuxe, 1964) (see Paper II).

The behaviour of the B-type fluorophore in the histochemical procedure is in many ways similar to that of the well-known monoamine fluorophores. Further, the spectral characteristics of the B-type fluorophore show interesting similarities to those of certain proposed biogenic indolyethylamines (Paper III), and are clearly different from those of the 5-hydroxytryptamine and catecholamine fluorophores. However, the identity of the compound that gives rise to the B-type fluorophore is not yet determined. Therefore it cannot be totally excluded that the B-type neurons contain 5-hydroxytryptamine or a catecholamine. If the intracellular conditions and/or dynamics of the B-type neurons in some way differ totally from those of the so far known monoamine neurons, these properties could possibly explain their deviating fluorescence characteristics and reactions to drug treatments. At all events, the differences in fluorescence characteristics, in reaction to drugs, and in topographical distribution between the B-type neurons, on one hand, and the A-type and C-type neurons on the other, seem to justify the recognition of the B-type neurons as a special and separate entity.

3. Sprouting from severed central monoamine neurons

Papers IV, V and VI

The previous studies devoted to the reactions of central monoamine neurons in response to injury have been concerned almost exclusively with the acute phenomena. Dahlström and Fuxe (1965) demonstrated that the monoamines accumulate intra-axonally within a few hours after cutting or traumatizing a monoamine nerve axon. In the spinal cord, these monoamine accumulations reached a maximum after about 24 hrs and persisted unchanged up to approximately the 12th day. Subsequently, they gradually disappeared (Dahlström and Fuxe 1965).

In the present studies (Papers IV, V and VI) a high capacity for the development of new axon sprouts has been demonstrated from electrolytically or mechanically severed axons in the mesencephalon and spinal cord of the rat. In the spinal cord, which has a morphological arrangement very suitable for observation of the growth and development of axon sprouts, the first traces of axon sprouting was observed as early as 2 to 3 days after compression of the spinal cord (Paper VI). The number of fine, varicose, fluorescent fibres with the appearance of new axon sprouts gradually increased. By the 10th to 11th postoperative day they were numerous, forming densely packed areas in the upper half of the necrotic zone. In the mesencephalon (Paper IV and V) the new fibres were seen to penetrate into the necrosis; and in the electrolytically lesioned animals, they formed plexa around the electrode tracks.

After survival times of two weeks or longer, numerous fine varicose fibres appeared within and along the walls of intracranial arteries and veins. This phenomenon was most frequent with the mesencephalic lesions, but it was occasionally found also with the spinal cord lesions. The abnormal fibre supply of the vessels was usually observed as densely packed, delicate, varicose structures distributed throughout the entire thickness of the wall, in contrast to the normals sympathetic innervation which is confined to the

adventitia.

The newly formed fibres in these various locations were found to be long lasting; and in some of the specimens, they retained the same abundance 6 months after the operation (Paper V).

To evaluate the possible contribution of sprouting sympathetic, peripheral fibres to the systems of newly formed catecholamine fibres, lesions were studied in animals where the intracranial blood vessels had been sympathectomized through a bilateral extirpation of the superior cervical ganglia. These experiments demonstrated that the sympathetic vascular fibres contributed only slightly, if at all, to the formation of the new fibres (Papers IV and V). In the spinal cord and in the animals carrying transplants in the mesencephalon, it was possible to trace newly-formed fibres back to the severed central monoamine axon bundles. (Paper V and VI).

The microspectrofluorometric analysis revealed axonal sprouting from noradrenaline, dopamine (A-type) and B-type neurons in the mesencephalon, and from noradrenaline (A-type) and 5-hydroxytryptamine (C-type) neurons in the spinal cord.

The catecholamine-containing fibre sprouts were most abundant and by far the most predominant after the mesencephalic lesions (Papers IV and V). With lesions in the spinal cord, the catecholamine-containing and the 5-hydroxytryptamine-containing sprouting fibres were approximately of the same abundance (Paper VI). The newly formed fibres had, to a certain extent, different morphological features. A clear morphological difference was thus observed between the dopamine-containing and the noradrenalin-containing fibres (Papers V and VI). The dopamin fibres formed an area of densely packed, delicate fibres around the severed axons in the medial forebrain bundle region. The newly-formed noradrenaline fibres were coarser, growing in a more loosely arranged fashion. The yellow-fluorescent sprouting fibres, i.e. the 5-hydroxytryptamine-containing and the B-type fibres, had a morphology rather similar to that of the dopamine-containing

fibres.

4. Growth of the newly-formed fibres into iris transplants

Papers V and VI

In order to demonstrate that the newly-appearing thin monoamine-containing fibres indeed were growing axonal sprouts, their ability to "re-innervate" iris transplants was tested.

It was found that the newly-appearing catecholamine fibres grew in abundance into the mesencephalic iris transplants. The ingrowing fibres originated from two main sources: from the area of initial abundant sprouting around the severed medial forebrain bundle axons, and from the lesioned axons in the so-called dorsal catecholamine tract. (Paper V). Both dopamine-containing and noradrenaline-containing fibres invaded the transplant. The dopamine fibres most probably originated in the medial forebrain bundle region, where the nigro-neostriatal dopamine pathway runs (Andén et al. 1966). The ingrowing noradrenaline fibres could, at least partly, be traced back to the dorsal catecholamine tract where noradrenaline-containing axons were identified microspectrofluorometrically.

The first fibres in the transplant were seen 7 days after transplantation, close to the area of initial sprouting in the region of the transected medial forebrain bundle. The new fibres had invaded the whole transplant 14-18 days after transplantation, and they still remained in abundance 6 months after operation. The experiments with sympathectomized animals revealed that the sympathetic adrenergic vascular fibres did not, to any observable extent, contribute to the fibres growing into the transplants.

The growth into the transplant had an interesting selectivity. Of the three different neuron types demonstrable with the Falck-Hillarp histofluorescence technique (Paper I and II), only the catecholamine-containing fibres grew into the transplant to any significant extent. Thus, no or only very few 5-hydroxytryptamine or B-type fibres were detected in the transplants.

It is rather improbable that this was due to a less potent sprouting capacity of these neuron types, as good sprouting was observed from the 5-hydroxytryptamine and the B-type axons at the border of the transplant in the spinal cord and in the mesencephalon, respectively. The transplantation experiments leave little doubt that the newly-appearing catecholamine fibres, observed upon electrolytic or mechanical lesions, are regenerating axon sprouts. The experiments performed in the spinal cord (Paper VI) clearly demonstrate that the sprouting occurs from the proximal stump of severed preterminal axons. No sprouting could be established from lesioned terminal or paraterminal axon parts. The sprouting capacity and the magnitude of the regenerative growth of the central monoamine neurons are remarkable and exceed those reported for any other neuron system in the adult mammalian central nervous system. This could well be due to a truly higher regenerative capacity of the central monoamine neurons. It is also possible, however, that the failure of other investigators to demonstrate significant regenerative growth from central neurons was due to the use of insufficiently sensitive methods.

5. Influence of the nature of the transplanted tissue on the growth of the newly formed catecholamine fibres

Paper V

Peripheral tissues receiving a varying degree and pattern of sympathetic, adrenergic innervation were transplanted to the medial forebrain bundle region in the rostral mesencephalon and caudal hypothalamus. They were examined fluorescence microscopically 14 to 18 days after operation.

The growth of newly formed central catecholamine fibres into the transplanted tissue appeared to be related to the degree of normal sympathetic innervation of the transplanted tissue. Thus, the iris transplants, which normally receive a rich sympathetic innervation, showed a large number of regenerating catecholamine fibres distributed throughout the iris tissue. After longer survival times, these fibres formed in places a pattern

much resembling that of the intact sympathetic innervation. In the mitral valve transplants the amount of ingrowing fibres was notably lower, and their distribution on the surface and pattern was similar to its normal sympathetic innervation. In contrast to this, the diaphragm and uterus transplants, which normally receive a slight adrenergic innervation, contained only few ingrowing fibres, apparently randomly distributed.

The results obtained in the presents experiments with "re-innervation", of peripheral tissues from central catecholamine neurons are to some extent comparable to those reported by Olson and Malmfors (1970) in the peripheral sympathetic nervous system. They studied the growth of sympathetic fibres into transplants placed in the anterior chamber of the eye. On the basis of a comparison between a number of different transplanted tissues, they concluded (p. 95) that the growth into the transplants of the sympathetic fibres was specific in the sense that the new nerves were largely confined to such transplanted tissues as are normally adrenergically innervated. Also, the new sympathetic nerves did not grow into areas where adrenergic nerves do not normally exist, and they did not grow into already innervated areas. Some of these conclusions seem valid also for the growth of central noradrenaline and dopamine neurons into peripheral transplants. This implies that the recipient tissue can determine the magnitude of growth and the organization of the regenerating axon sprouts also in the central nervous system.

6. Plastic changes in the adrenergic innervation of the rat septal area in response to denervation

Paper VII

The pronounced capacity for regenerative sprouting from severed central catecholamine neurons suggested the possibility that these neurons could be capable of exhibiting growth also in the intact state. Such growth or sprouting from intact neurons might form a basis for a morphological

plasticity of the nervous system. There is, in fact experimental evidence that intact central axons may grow to re-innervate an area partially denervated by removal of a separate source of afferent input (Liu and Chambers 1958, Ma Couch et al. 1958, Goodman and Horel 1967, Raisman 1969). In a recent study, Raisman (1969) obtained evidence suggesting that medial forebrain bundle axons can grow to re-innervate medial septal nucleus neurons that have been partially denervated by section of the fimbria. Raisman's observations were taken to indicate that vacant synaptic sites in the denervated medial septal nucleus could be occupied by sprouting medial forebrain bundle axons. The validity of this conclusion, however, depends largely on the reliability of the relative frequency of multiple and single contacts as an indicator of synaptic reorganization, as used by Raisman. The present study was undertaken to make a direct study of the capacity for growth of intact catecholamine axons in the partially denervated septum.

The adrenergic innervation of the rat septal area was studied with the Falck-Hillarp histofluorescence method in normal animals and in those whose septum had been denervated by an unilateral hippocampal lesion. In the normal septum, adrenergic terminals were found in all the principal septal nuclei with the heaviest innervation apparent in the lateral septal nucleus, the nucleus accumbens, and the interstitial nucleus of the stria terminalis. After a total, unilateral transection of the hippocampal fimbria, there was a marked increase in the adrenergic innervation of those septal nuclei that receive a significant projection from the hippocampus. This first appeared between 8 and 15 days after operation, became marked by 30 to 60 days, and persisted for at least 100 days after the septal denervation. The increase in the number of adrenergic terminals was initially found in the caudal part of the lateral septal nucleus, and subsequently, it was evident through most of the lateral septal nucleus, the medial septal nucleus, and the posterior septal nucleus. No changes were noted in the other nuclei of the septal area. Concomitant with the histochemically

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demonstrable increase in adrenergic innervation, there was a biochemically detectable increase in the noradrenaline content of the septum. The source of the adrenergic innervation in both the normal and denervated septum appears to be axons traversing the medial forebrain bundle, as transection of this tract produces a substantial reduction in the adrenergic innervation of the septal nuclei.

The most attractive, and also the most probable, interpretation of these results is that the histochemically demonstrable increase in the density of adrenergic terminal innervation in the septal nuclei represents a true increase in the number of such terminals. This thus implies that the noradrenaline axons normally innervating these nuclei have sprouted to produce new terminals re-innervating synaptic sites denervated by the hippocampal lesion. There are alternative interpretations, however; these are discussed at length in Paper VII.

These findings, and studies on other central monoamine neuron systems (Björklund, Moore and Stenevi, to be published) indicate that sprouting from intact and severed monoamine neurons is a not unusual phenomenon in the rat brain. This should be taken into account in studies on the effects of lesions in the central nervous system.

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